IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Peter SCHUBERT et al.

Serial No.: 08/075,248
Filed November 06, 1993
For: PROCESSES AND AGENTS FOR DETECTING LISTERIAS

Group Art Unit: 1806
Examiner: R. SCHWADRON

DECLARATION

Honorable Commissioner of
Patents and Trademarks
Washington, D.C., 200231

SIR:

The Declarant, Siegfried Neumann, being duly warned, declares and says:

THAT he is a German citizen, residing at Seeheim-Jugenheim, Germany;

THAT he is a biologist by training and experience;

THAT he studied biology and biochemistry at the University of Mainz, Germany, from 1962 to 1968, at the University of Frankfurt, Germany, from 1968 to 1969, and at the University of Bochum, Germany, from 1969-1971;

...

hg 4040 6 R

THAT he graduated from the University of Mainz in 1968;

THAT he obtained the Dr. rer. nat. degree from the University of Bochum in 1971;

THAT he worked as postdoctoral research associate at the University of Bochum from 1971 to 1975;

THAT, in 1975, he joined the Research and Development Department, Biochemical Department, of E. MERCK, Darmstadt, Germany;

THAT, since 1968, he has been working in the field of immunology and immunologic testing;

THAT he is author or co-author of numerous articles in the field of immunology and immunologic testing;

THAT he is inventor or co-inventor of numerous inventions in the field of immunology and immunologic testing;

THAT he is familiar with the subject invention disclosed and claimed in U.S. Patent Application Ser. No. 08/075,248, filed November 06, 1993, by Peter Schubert et al. (hereinafter referred to as APPLICATION), of which he is co-inventor;

THAT he is familiar with the subject matter disclosed in the cited references;

THAT he studied the review of R.A. Lerner (1984) Advances in Immunology **36**, 1-44, cited by the Examiner, which is based on experimental data related to influenza virus hemagglutinin published by N. Green et al. (1982) Cell **28**, 477-487, as well as the papers by A. Nestorowicz et al. (1985) Molecular Immunology **22**, 145-154, and by M.H.V. van Regenmortel et al. (1988) in "Laboratory Techniques in Biochemistry and Molecular Biology", Vol. **19**, chapter 4.3, pages 139-141;

THAT the basic assumption of Lerner is that practically all peptides derived from the sequence of a given protein give rise to antibodies which react with said protein;

THAT this view was disapproved by A. Nestorowicz et al. (1985) (see page 152, lower half of left column);

THAT A. Nestorowicz et al. point out that it is difficult to make any generalisations about the requirements necessary for immunogenic activity of a given peptide and that the proportion of peptide molecules existing in the conformation most representative for the native conformation of protein may be as little as 10^{-4} -10^{-5} (see pages 151-152);

THAT the view of A. Nestorowicz et al. (1985) is independly supported by M.H.V. van Regenmortel et al. (1988), who state that the conformation, which will be adopted by a peptide, cannot be predicted in a reliable manner, and that therefore an empirical approach is best in order to obtain the antibodies desired (see page 141);

THAT consequently the opinion expressed by Lerner is by no means a dominating opinion within the scientific community;

THAT the above findings are corroborated by the experimental data submitted in the Declaration by P. Schubert;

THAT these results are surprising in view of his earlier experience and not predictable by a person having ordinary skill in the art;

THAT the undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the APPLICATION or any patent issuing thereon.

Done, this April 13, 1994 at Darmstadt, Germany

Siegfried Neumann

Siegfried Neumann

; (Palfreyman et al., 1984), the general
ıntibodies against proteins should be
Crumpton, 1974; Mayer and Walker,
).

igens with a molecular weight smaller
and are not immunogenic. This belief
ice of immunizing animals with pep-
ule. There are indeed many reports in
ıunization with conjugated peptides
ı immunization with the correspond-
1985; Choppin et al., 1986; Schulze-
1986).

ɔoratory have shown that immuniza-
ı of 14–25 residues (using protocol
tibody response after 2–3 injections
ain antisera of adequate titre, 5–6 in-
me authors have reported that satis-
ɔtained by immunizing rabbits and
of 6–8 residues (Young et al., 1983;
al., 1985; Young and Atassi, 1985).
› raise antibodies by immunization
ıg to fragments of histone molecules

antipeptide antibodies is usually to
with the parent protein, the success
ıs on the cross-reactive potential of
titre measured with respect to the
ɔen a conjugated peptide is used for
:arrier molecule may induce a con-
lose to that present in the parent
:ntipeptide antibodies would be ex-
activity with the complete protein
another method used for making
f the corresponding segment of the
'clized peptides has been found to

lead to antisera possessing a high degree of cross-reactivity with the intact protein (Dreesman et al., 1982; Dorow et al., 1985; Kanda et al., 1986).

As the length of a peptide fragment increases, the likelihood that its conformation will resemble that found in the corresponding part of the complete protein also increases. This may explain why longer peptides react better with antiprotein antibodies than do shorter peptides, and why they also induce antibodies that cross-react more strongly with the parent protein (Van Eldick et al., 1983; Dorow et al., 1985; Welling and Fries, 1985; Tanaka et al., 1985; Al Moudallal et al., 1985; Van Regenmortel et al., 1986). However, it may be impossible to predict for any particular peptide whether conjugation to a carrier or cyclization is likely to lead to a structure that better mimics the conformation found in the native protein. For many years, it was commonly assumed that free peptides could adopt a very large number of different conformations in aqueous solution (see chapter 1). However, recent experimental observations show that this is not always the case and that peptides may in fact adopt a few preferred conformations (Dyson et al., 1985). The extent to which any peptide is able to mimic a conformation present in the intact protein depends, in the last analysis, on its individual sequence. Since we cannot yet predict, in a reliable manner, the conformation which a peptide is likely to adopt, it is not possible to state which immunization approach is most likely to succeed in any particular instance. It seems best to follow an empirical approach and to test a variety of immunization procedures using free, conjugated and cyclized peptides in succession until the desired antibodies are obtained.

4.4. Immunization with conjugated peptides

Several protein carriers are commonly used to obtain peptide conjugates suitable for immunization (see Table 3.3). The use of highly immunogenic substances such as KLH can be detrimental in certain cases, probably because of antigenic competition phenomena (Taussig, 1971; Sarnesto et al., 1983). In the case of peptides that are only poorly im-

M.H.V. van Regenmortel et al. (1988)